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Tuber sinosphaerosporum sp. nov. from China

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Abstract — A new white truffle species from China is described and illustrated. *Tuber sinosphaerosporum* is characterized by its white ascomata and globose ascospores ornamented with large reticulum meshes. The new species, supported by ITS sequence analysis, offers commercial value due to its moderate to relatively large size and possibly high yield in China.

Key words —*Ascomycota*, taxonomy

Introduction

The number of *Tuber* species in China has increased considerably over the last thirty years (Liu 1985, Wang 1988, Wang & Li 1991, Wang et al. 1998, Wang & He 2002, Tao et al. 1989, Hu 1992, Xu 1999, He et al. 2004, Hu & Wang 2005, Chen et al. 2005, Chen & Liu 2007, Cao 2010, Fan et al. 2011, 2012a,b), and new species are still being found. In December 2010, about a kilo of fresh white truffles growing under *Corylus* sp. was collected from Baoshan City of Yunnan Province; they had a strong but pleasant aroma of garlic, typical of white truffles. Subsequently, another white truffle was collected from Chenggong County near Kunming City of Yunnan that was morphologically similar to the Baoshan specimens but grew under conifers. Detailed morphological observation and molecular research confirm that the collections from both locations represent a single undescribed species.

Materials & methods

Morphological studies

Truffles were collected from Baoshan City and Chenggong County, Yunnan Province. Macroscopic characters were described from fresh specimens. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 3% KOH,

Table 1 *Tuber* specimens and sequence numbers used in molecular studies.

Species name	Voucher	Origin	ITS	Reference
T. borchii Vittad.	GB62	Italy	HM485342	Bonito et al. 2010
	GB39	Unknown	HM485343	Bonito et al. 2010
	GB45	Italy	HM485344	Bonito et al. 2010
T. borchii var. *sphaerospermum* Malençon	HKAS 520005	China	GQ 217541	GenBank
T. californicum Harkn.	L4AB7	Unknown	EF411102	Morris et al. 2008
	JT22590	USA	HM485351	Bonito et al. 2010
	JT28058	USA	HM485346	Bonito et al. 2010
T. latisporum Juan Chen & P.G. Liu	HKAS 30838B	China	DQ898185	Chen & Liu 2007
	HKAS42380	China	DQ898184	Chen & Liu 2007
	HKAS44315	China	DQ898183	Chen & Liu 2007
T. melanosporum Vittad.	A59	France	AF106878	From GenBank
	TM13	France	AF132501	Roux et al. 1999
T. oligospermum (Tul. & C. Tul.) Trappe	MA: FUNGI: 41010A	Spain	FM205506	GenBank
	MA: FUNGI: 41010B	Spain	FM205507	GenBank
	MA: FUNGI: 28388B	Spain	FM205508	GenBank
	MA: FUNGI: 28389	Spain	FM205509	GenBank
T. puberulum Berk. & Broome	TL11885	Denmark	AJ969626	Tedersoo et al. 2006
	TL3857	Denmark	AJ969625	Tedersoo et al. 2006
	BI-32	Hungary	AJ557537	Halász et al. 2005
T. sinosphaerosporum	BJTC FAN135 (HOLOTYPE)	China	JX092086	This study
	BJTC FAN136	China	JX092087	This study
T. sphaerosporum Gilkey	JT12487	USA	FJ809853	Bonito et al. 2010
	JT19772	USA	FJ809854	Bonito et al. 2010
	JT12487	USA	GQ221449	From GenBank
	OSC75864	USA	HM485390	From GenBank

Melzer's reagent, or 0.1% (w/v) cotton blue in lactic acid. The specimens are deposited in BJTC (Biology Department Herbarium, Capital Normal University). For scanning electron microscopy (SEM), spores were scraped from the dried gleba, placed on to doubled-sided tape, mounted directly on an SEM stub, coated with gold–palladium, and then examined and photographed with a HITACHI S-4800 SEM.

Molecular methods

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm diameter tungsten carbide ball. Total genomic DNA was then extracted using the PeqLabE.Z.N.A._Fungal DNA kit following the manufacturer's protocol. The ITS region was amplified with PCR using the primers ITS1/ITS4 (White et al. 1990). PCR was performed in 50 µl reactions containing DNA template 2 µl, primer (10µM) 2 µl each, 2 × Master Mix (Tiangen Biotech (Beijing) Co. Ltd.) 25 µl. PCR reactions were run as follows: an initial

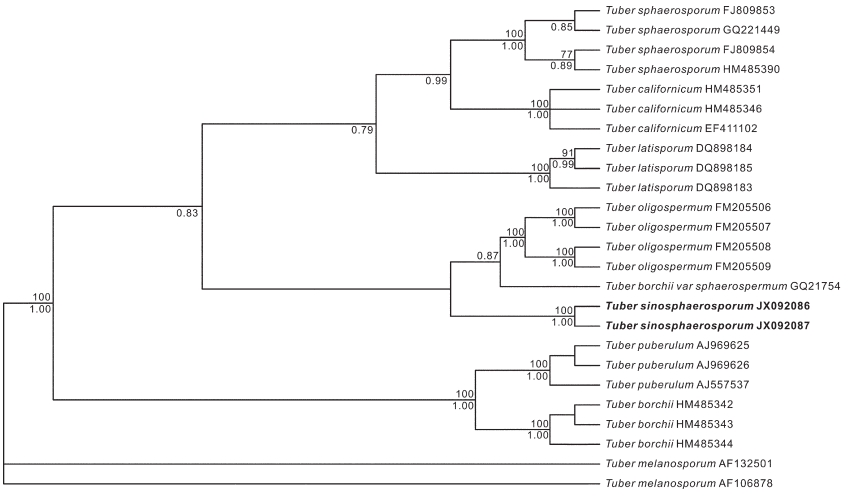

Fig. 1. Phylogeny derived from maximum parsimony ITS rDNA sequence analysis of some *Tuber* species with reticulate ascospore ornamentation using *T. melanosporum* as outgroup. Bootstrap values of >70% from 1000 replications are shown above the respective branches. Clades with Bayesian posterior probabilities (PP) estimated >0.70 (70%) are marked under the branches.

denaturation at 95°C for 3 min, followed by 30 cycles at 95°C for 2 min, 55°C for 25 s, 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data of ITS rDNA included in this study were downloaded from GenBank. GenBank numbers are shown in Table 1.

Phylogenetic analyses

DNA sequences were aligned with Clustal X (Thompson et al. 1997). The alignment was manually adjusted with Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002). Maximum parsimony analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed. The Bayesian analysis was performed with MrBayes 3.1.2 (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003) with two sets of four chains (one cold and three heated) and the stoprule option in effect, halting the analyses at an average standard deviation of split frequencies of 0.01. The sample frequency was set to 100, and the first 25% trees were removed as burn-in. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of the remaining trees. Two sequences derived from *Tuber melanosporum* were used as outgroup.

Results

Molecular phylogenetics

279 of 612 characters were found to be parsimony-informative. Maximum parsimony analysis resulted in one most parsimonious tree (Fig.1) with a length (TL) of 510 steps, consistency index (CI) of 0.7804, retention index (RI) of 0.8948 and rescaled consistency index (RCI) of 0.6983 (for all sites).

The ITS sequence phylogeny (Fig. 1) revealed that sequences from the two Yunnan collections (*Tuber sinosphaerosporum*) were the same and grouped in a clade with strong support (BS = 100, PP = 1.00). They appear to form a sister relationship with *T. borchii* and *T. oligospermum* but with very low support.

Taxonomy

Tuber sinosphaerosporum L. Fan, J.Z. Cao & Yu Li**, sp. nov.** Fig. 2

MycoBank MB800677

Differs from other *Tuber* species in its fresh white ascomata and regular globose ascospores covered by a reticulate ornamentation that is 2–4 meshes across the spore width.

Type: China. Yunnan Province, Baoshan City, under the soil near *Corylus* sp. 11 Dec. 2010, De-fu Liu (**Holotype**, BJTC FAN135; GenBank, JX092086))

Etymology: *sinosphaerosporum* (Lat.), referring to a Chinese species resembling the American *Tuber sphaerosporum*.

Ascomata irregularly globose or lobed, often convolute and with several deep furrows, 1.5–5.5 cm in diam., white or whitish-cream or pale yellow white when fresh, yellow whitish or light yellow brown after dried, the surface poorly puberulent at least at furrows. Odor faint when young, but of strong garlic when mature. Peridium 250–300 μm thick, with two layers: outer layer 100–150 μm thick, pseudoparenchymatous, composed of subglobose and angular cells 7.5–17.5 μm in diam., with slightly thickened walls, yellowish-brown towards the surface; inner layer 150–200 μm thick, texture intricate, the hyphae thin-walled, hyaline, 2.5–5 μm in diam. Hairs hypha-like, arising from the superficial cells in places, 10–40 × 2.5–5(–7.5) μm, hyaline or yellowish, usually thin-walled, 1–2 septate, cylindric and obtuse at the apex. Gleba white at first, becoming yellow-brown to brown at maturity, marbled with large and rare, branched, white veins originating from various points of the inner peridium. Asci globose, subglobose or broadly ellipsoid, sessile, 75–125 × 62.5–85 μm, 1–4-spored. Ascospores regularly globose, hyaline at first, becoming yellow brown to brown at maturity, ornamentation regular reticulum, 20–42.5 μm in 2–4-spored asci and 40–45 μm in 1-spored asci in diam. excluding the ornamentation, the meshes large and variable in size, 5.0–7.5 μm high, usually 2–4 meshes across the spore width.

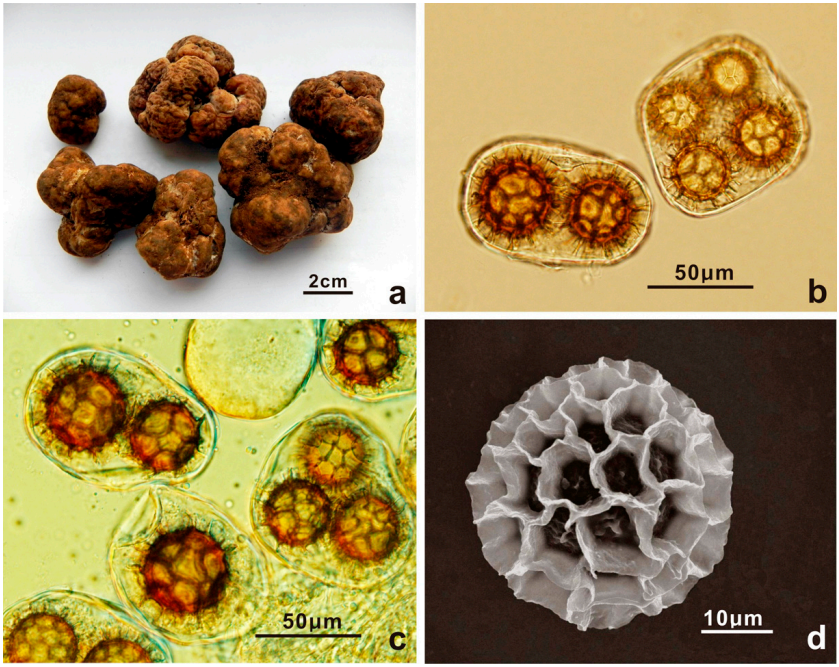

Fig. 2. *Tuber sinosphaerosporum* (BJTC FAN135, holotype). a. Soil adhering to the ascomal surface makes them appear brown; b–c. Asci and ascospores observed under light microscope; d. Ascospore observed under SEM.

Additional specimens examined: China, Yunnan Province, Chenggong County, under soil of conifers, 18 Dec. 2010, Jin-zhong Cao (BJTC FAN136; GenBank, JX092087).

Comments — *Tuber sinosphaerosporum* is similar to American *T. sphaerosporum* in the appearance of ascospores, but the American species differs in its glabrous brown ascomata and dark gleba (Gilkey 1939, 1954). The peridium of *T. sphaerosporum* is composed of "variable cells, the large and small intermixed, somewhat pseudoparenchymatous or often prosenchymatous" (Gilkey 1954), whereas *T. sinosphaerosporum* has typical pseudoparenchymatous peridium. The molecular study (Fig. 1) also demonstrated a considerable separation.

The other *Tuber* species with regular globose ascospores, such as *T. californicum* from North America, *Tuber borchii* var. *sphaerospermum* from Europe, and *T. oligospermum* from Europe and North Africa, are distinguished from *T. sinosphaerosporum* by their reticulum meshes numbering 5–7 across the ascospore width (Gilkey 1939, 1954, Riousset et al. 2001). Phylogenetic analyses (Fig. 1) support *T. sinosphaerosporum* as a distinct species. The

T. sinosphaerosporum sequences form a sister relationship with *T. borchii* and *T. oligospermum,* but the very low support value indicates they are closely related but distinct.

Tuber sinosphaerosporum could be a potential commercial species in the international white truffle market because of its significant larger ascocarp size (medium to relatively large) and strong but pleasant aroma. According to the local people, *T. sinosphaerosporum* could be expected to have a high cropping yield in Yunnan Province.

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